Physical and Chemical Characterization of an Oyster Hemagglutinin*

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SUMMARY

The natural hemagglutinin of the oyster Crassostrea virginica was examined for possible structural relationship with vertebrate immunoglobulins. The hemagglutinin activity was associated with a heterogeneous group of rapidly sedimenting molecules. The major active component in oyster hemolymph has a sedimentation coefficient of 33.4 S, but minor components with lower sedimentation coefficients are also present. The molecules can be dissociated into subunits consisting of single polypeptide chains having an approximate molecular weight of 20,000. Complete dissociation could be observed in 5 M guanidine-HCl indicating non-covalent linkage of the subunit. Amino acid composition of the subunit was distinguished by a relatively high histidine content and the absence of lysine. The sequence of the first 2 residues from the NH₂ terminus of the subunit was found to be Thr-Ala. Mannose, galactose, and glucosamine were present with a total carbohydrate content of 13%. These data do not demonstrate obvious structural similarities to mammalian immunoglobulins.

A number of investigators have shown structural similarities between the light and heavy polypeptide chains of immunoglobulins (1-7). From sequence data, Hill et al. (8) have proposed a hypothetical scheme for the evolutionary origins of immunoglobulins. In this scheme the genes coding for immunoglobulin light and heavy chains are considered to have evolved from a common ancestral gene by a process of duplication and mutation. The light and heavy chains found in the sera of vertebrates.

EXPERIMENTAL PROCEDURE

Materials

Hemolymph was collected from the pericardial sinus of live oysters as previously described (19). After removing the cells by centrifugation the hemolymph was pooled and stored at -25°C. Guanidine hydrochloride (reagent grade, Matheson Coleman and Bell) was purified by repeated passage of a 7 M solution through a charcoal column (65 x 6 cm, bed volume of 1500 ml) until the optical density at 250 nm was below 0.05 per cm. Refractive index measurements were made on an Abbe refractometer from which concentrations and densities were determined as described by Kielly and Harrington (20).

Sephadex G-200 (Pharmacia) was prepared for chromatography by boiling for 3 hours in distilled water. After cooling, the fine particles were removed by placing 800 ml in a 2-liter separatory funnel and allowing water to flow slowly upward percolating the fine beads out the top. The gel was then equilibrated with 5 M guanidine hydrochloride, pH 5 ± 1, for 3 to 4 days before column preparation.

Sephadex 4B (Pharmacia), obtained as swollen beads in distilled water, was washed with 0.01 M Tris(hydroxyxymethyl)aminomethane buffer, pH 7.4, containing 0.01 M of the tetrasodium salt of ethylenediaminetetraacetate and 0.14 M NaCl. The washed gel was allowed to equilibrate overnight with the buffer before being poured into a column.

Sheep erythrocytes were obtained preserved in Alsevers solution from Colorado Serum Company, Denver, Colorado. Tryp-
sin and chymotrypsin were purchased from Worthington and stored in 0.001 N HCl at a concentration of 10 mg per ml. Anthrone and reagent grade Tris were obtained from Sigma. The tetrasodium salt of EDTA was obtained from Nutritional Biochemicals. Acrylamide gelling agents and catalyst were obtained from Fisher. N-Ethylmorpholine, iodoacetamide, 2-mercaptoethanol, and phenyl isothiocyanate were obtained from Eastman. All solvents used in the Edman procedure were redistilled. All naphthalenesulfonyl chloride was obtained from the Calbiochem. Other materials used were reagent grade according to American Chemical Society standards.

Methods

Activity Measurements—Hemagglutinin activity was assayed by tube agglutination test as outlined by Kabat and Mayer (21). Sheep erythrocytes were washed free of plasma and suspended in 0.85% sodium chloride solution containing 0.001 M Mg++ and 0.000015 M Ca++ (Mg++-Ca++ sodium chloride solution) as 1% (v/v) cell suspensions. The 2-fold serial dilutions were prepared, incubated at 37° for 2 hours and left overnight at 5° before reading. The last tube having visible agglutination was the dilution titer recorded.

Purification of Oyster Hemagglutinin—Hemolymph was fractionated by gel filtration at 10° utilizing upward-flowing Sepharose 4B columns (2.5 x 100 cm; bed volume, 480 ml; flow rate 15 ml per hour) equilibrated with 0.01 M Tris, 0.01 M EDTA, 0.14 M NaCl buffer, adjusted to pH 7.4 with HCl. Immediately prior to gel filtration oyster hemolymph was thawed at room temperature, concentrated by pressure dialysis, and dialyzed against the Tris-EDTA-NaCl, pH 7.4, buffer at 5°. Fractions of 5 ml each were collected and monitored by absorption at 280 M. Pooled fractions were concentrated by pressure dialysis and dialyzed against Mg++-Ca++ sodium chloride solution at 5° before assaying for hemagglutinin activity. The fraction containing hemagglutinin activity was recycled through Sepharose 4B. The final product (purified hemagglutinin) was exhaustively dialyzed against water and lyophilized.

Electrophoresis—Disc electrophoresis in polyacrylamide gel was adapted from the procedure of Maizel (22). A solution containing 5% acrylamide, 0.13% N,N'-bis-methylhexacycliamide, 0.1 M sodium phosphate (pH 7.2), 0.1% sodium lauryl sulfate, and 0.5 M urea was prepared in distilled water. Polymerization was catalyzed by adding 1 ml of freshly prepared 10% ammonium persulfate and 30 µl of N,N',N',N'-tetramethylthelylene to 60 ml of solution. Gel columns (7 cm long and 6 mm in diameter) were filled with the final solution, layered with distilled water, and allowed to gel for 1 hour. The buffer chamber contained 0.1 M sodium phosphate (pH 7.2) and 0.1% sodium lauryl sulfate. Three millamps per tube were applied for 4 hours. Gels were stained with 1% Amido black in 7% acetic acid. Destaining was carried out by several changes of 40% methanol in 10% acetic acid.

Reduction and Alkylation—Total reduction was carried out by dissolving purified oyster hemagglutinin in 7 M guanidine hydrochloride, adjusted to pH 7.6 by the addition of 0.5 M Tris-HCl, followed by the addition of 0.2 M 2-mercaptoethanol. The mixture was incubated at 37° for 4 hours. Alkylation was achieved by adding a slight molar excess of iodoacetamide and incubating at room temperature for 15 min. Totally reduced and alkylated material was applied to a Sephadex G-200 upward-flowing column (2.5 x 120 cm) equilibrated with 5 M guanidine hydrochloride as described by Small and Lamm (23) with flow rates of 10 ml per hour. After monitoring the column effluent at 280 M, indicated fractions were exhaustively dialyzed against distilled water and lyophilized. Partial reduction was carried out by dialysis against 0.1 M 2-mercaptoethanol, in 0.1 M sodium phosphate buffer, pH 7.5, for 3 hours at room temperature. Alkylation was achieved by dialysis against 0.02 M iodoacetamide, prepared in the same buffer, for 4 hours at room temperature. Samples were then dialyzed overnight against Mg++-Ca++ sodium chloride solution, pH 7.0, at 5°.

Ultracentrifugation—Sedimentation velocity experiments were performed at 20° in a Spinco Model E ultracentrifuge with single-sector 12-mm cells, sliver optics, phase plate angle of 60°, and a speed of 56,100 rpm. The patterns were measured on a Nikon micro comparator. Sedimentation coefficients were calculated according to procedures described by Schachman (24) and corrected to the reference state of water at 20°. The partial specific volume was assumed to be 0.73.

Sucrose density gradient ultracentrifugation was performed according to the methods of Kunkel (25). A 10 to 40% continuous gradient of sucrose was prepared in Mg++-Ca++ sodium chloride solution and samples were run for 16 hours with an SW

![FIG. 1. Sucrose density gradient ultracentrifugation experiments showing the distribution of oyster hemagglutinin reactive against sheep erythrocytes. Vertical bars represent hemagglutination titers. A, whole oyster hemolymph; B, purified oyster hemagglutinin partially reduced and alkylated (RED-ALK); C, markers consisting of human 19 S and 7 S immunoglobulins.](http://www.jbc.org/)

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Fig. 2. Ultracentrifugation patterns of whole and purified oyster hemolymph in 0.1 M sodium phosphate, pH 7.5. The photographs were taken after 16 min at 56,106 rpm at a phase plate angle of 60° and temperature of 20°. A, whole oyster hemolymph (protein concentration, 5.5 mg per ml); B, purified oyster hemagglutinin (protein concentration, 2.8 mg per ml). Sedimentation proceeds from left to right.

Fig. 3. The concentration dependence of the sedimentation coefficient ($s_{20,W}$) for purified oyster hemagglutinin (HA) in 0.1 M sodium phosphate buffer, pH 7.5. The extrapolated value of $s_{20,W}$ at infinite dilution is indicated in parentheses.

Fig. 4. Elution pattern of oyster hemolymph from a Sepharose 4B column (2.5 X 106 cm) equilibrated with 0.01 M Tris-HCl, 0.01 M EDTA, 0.14 M NaCl, pH 7.4. Analytical acrylamide gel electrophoresis patterns superimposed on the elution pattern illustrate the electrophoretic pattern of the indicated pool. The anode is to the bottom of the figure. The area under the cross bar illustrates the volume pooled. A, whole oyster hemolymph; B, Pool 1 from A recycled.

Carbohydrate Determinations—Total hexose was determined by the anthrone method of Bailey (27) on weighed samples of lyophilized protein with galactose as a standard. Hexoses and hexosamines were identified by hydrolyzing 10 mg of protein in 2 N HCl for 15, 20, 120 min in a sealed, evacuated ampule at 110°. The excess HCl was removed by evaporation under reduced pressure on a rotary evaporator. The hydrolysates were dissolved in 0.5 ml of water and spotted on Whatman No. 1 chromatography paper with appropriate standards. Descending chromatography was carried out in 1-butanol-pyridine-water (5:3:2) for 20 and 40 hours. The separation was checked by additional descending chromatography of hydrolysates in ethyl acetate-pyridine-water (12:5:4). The sugars were detected by the
modified silver nitrate spray methods of Trevelyan, Procter, and Harrison (28). Blanks and standards were treated in an identical manner throughout the procedures. Hexosamine was determined on the Beckman model 120C automatic amino acid analyzer. Samples were hydrolyzed in 6 N constant boiling HCl under vacuum at 106°C for 2, 4, and 8 hours. After hydrolysis, excess HCl was removed in a vacuum over NaOH.

Amino Acid Analysis—Samples were hydrolyzed in 6 N constant boiling HCl under vacuum at 106°C for 20 hours. After hydrolysis excess HCl was removed in a vacuum over NaOH. Analyses were performed on the Beckman model 120C automatic amino acid analyzer with a 55-cm column for separation of neutral and acidic amino acids and an 8-cm column for basic amino acids as described by Hubbard (29). Tryptophan was determined spectrophotometrically by the N-bromosuccinimide method of Spande and Witkop (30).

Peptide Maps—Peptide maps were prepared as described by Bennett (31). Protein samples (10 mg per ml) were digested separately with trypsin and chymotrypsin at 37°C for 4 hours in 0.1 M NH₄HCO₃ (pH 8.2). Approximately 1.0 to 1.5 mg of protein were spotted on Whatman No. 3MM chromatography paper and subjected to descending chromatography for 20 hours. The organic phase of 1-butanol-glacial acetic acid-water (4:1:5) was used as the chromatographic solvent. Vapor equilibrium was achieved by placing the aqueous phase in the bottom of the chamber. Electrophoresis in the second dimension was in pyridine acetate buffer (100 ml of acetic acid and 10 ml of pyridine diluted to 3000 ml), pH 3.6, at 3200 volts for 65 min at 22°C in a Gilson Medical Electronic model DW electrophorator. The papers were dried for 20 min in an oven at 80°C. Peptide maps were performed in duplicate for each digest. One map was stained with ninhydrin-collidine and the remaining map sprayed with the Ehrlich stain on one side to detect tyrosine- and histidine-containing peptides, and then dipped in Ehrlich reagent to detect tryptophan-containing peptides.

Sequence Analysis—The Edman degradation method was carried out by using the three cycle modification as described by Edman (32). After extraction with ethylene chloride the thiazolidones were converted to free amino acids, dansylated according to the method of Gray (33), and identified by his electrophoretic system utilizing a Savant flat plate. As an additional check, the thiazolidones were converted to the phenylthiohydantoin derivative and detected by chromatography according to the method of Sjöquist (34). The cyanate method of Stark (35) was used to determine the NH₂-terminal group quantitatively.

**RESULTS**

Several physical chemical characteristics of oyster hemolymph were investigated to determine whether molecules were present which had any relationship to vertebrate immunoglobulins. Our activity studies confirmed those of Tripp (18) and Mcdade and Tripp (36) in that the hemagglutinin factor was found to be
The ultrafiltration provided more exact data on the size of molecules in oyster hemolymph. The sedimentation pattern of whole oyster hemolymph in 0.1 M sodium phosphate, pH 7.5, is shown in Fig. 21. The major component at this concentration (5.5 mg per ml) sedimented at 29 S. Also present were minor components sedimenting at 26, 9, and 8 S. Similar results were obtained on analytical ultracentrifuge analysis of whole oyster hemolymph run in Mg++-Ca++ sodium chloride solution, pH 7.0. The major component in oyster hemolymph had a sedimentation constant of 33.4 S at infinite dilution. The sedimentation was shown to be concentration dependent (Fig. 3).

Gel filtration was utilized in attempts to purify the component or components of oyster hemolymph responsible for hemagglutination. Oyster hemolymph was concentrated to approximately 50 mg per ml and 5 ml were applied to an upward-flowing Sepharose 4B column. The concentrate had a hemagglutinin titer of 1:64. Separation into two peaks was obtained (Fig. 4A) with hemagglutinin activity associated only with the first peak eluted (Pool 1). When concentrated to 0 ml, Pool 1 had a hemagglutinin titer of 1:64. Pool 2, however, contained a larger quantity of component than expected, based on the ultracentrifugal analysis of the unfractionated hemolymph (Fig. 2A). This suggests that dissociation may have occurred during gel filtration. This is also consistent with the absence of hemagglutinating activity since, as shown below, dissociation of the macromolecular components is accompanied by a loss of this property. Also shown in Fig. 4A are polyacrylamide gel electrophoresis patterns of the two pools. As shown from the electrophoretic profiles, essentially the same components, based on size and charge, were present in both pools. However, Pool 2 contained more of the lighter, positively migrating material. Pool 1 from several runs on Sepharose 4B was concentrated and recycled. Examples of the elution pattern and the polyacrylamide electrophoresis patterns of the pool are shown in Fig. 4B. The pool had a hemagglutinin titer of 1:128 and had the same acrylamide electrophoretic components as in Pool 1 (Fig. 4A). When the pool from Fig. 4B was analyzed in the ultracentrifuge (Fig. 2B), at a concentration of 2.8 mg per ml, the major component had a sedimentation coefficient of 29 S. Also present were minor components having sedimentation coefficients of 27, 24, and 18 S which represented a larger percentage of the hemolymph proteins than seen in the original material (Fig. 2A). This pool will be referred to as purified hemagglutinin and material used in further experiments was prepared in this manner. These chromatographic data would indicate that the hemagglutinin is associated with a heterogeneous group of molecules. Since there was evidence that these components would aggregate and dissociate under various conditions of ionic strength and pH, studies were carried out to determine whether the major components in oyster hemolymph were made up of subunits and if so, the manner in which the subunits were bonded together.

Some of the ultracentrifugal sedimentation characteristics of purified oyster hemagglutinin in various solvents are listed in Table I. Purified hemagglutinin had essentially the same sedimentation pattern in Mg++-Ca++ sodium chloride solution, pH 7, or in 0.1 M phosphate buffer, pH 7.0 (Experiments A and B). The major components in both solvents sedimented with an oberved sedimentation coefficient of 29 S. Whole oyster serum had a pH of 7.4 and the purified hemagglutinin was found to be stable within a pH range of 7 to 8 while retaining hemagglutinin activity. If the pH was changed to the alkaline or acidic side.

### Table I

<table>
<thead>
<tr>
<th>Experiment, procedure, and solvent</th>
<th>pH</th>
<th>Sedimentation characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Principal component</td>
</tr>
<tr>
<td>A. Untreated: 0.85% NaCl solution + 0.00015 M Ca** + 0.001 M Mg**</td>
<td>7.0</td>
<td>29.8</td>
</tr>
<tr>
<td>B. Untreated: 0.1 M sodium phosphate</td>
<td>7.5</td>
<td>29.3</td>
</tr>
<tr>
<td>C. Untreated: 0.14 M NaCl + 0.01 M Tris-HCl</td>
<td>9.6</td>
<td>2.9</td>
</tr>
<tr>
<td>D. Untreated: 0.2 M glycine-HCl</td>
<td>3.6</td>
<td>12.4, 4.5</td>
</tr>
<tr>
<td>E. Partially reduced and alkylated: 0.1 M sodium phosphate</td>
<td>7.5</td>
<td>25.3</td>
</tr>
<tr>
<td>F. Partially reduced and alkylated: 5 M guanidine-HCl</td>
<td>7.5</td>
<td>4.8</td>
</tr>
<tr>
<td>G. Untreated: 5 M guanidine-HCl</td>
<td>7.5</td>
<td>0.9</td>
</tr>
<tr>
<td>H. Totally reduced and alkylated, purified hemagglutinin, subunit: 5 M guanidine-HCl</td>
<td>5.0</td>
<td>21.1, 13.1, 12.0</td>
</tr>
<tr>
<td>I. Untreated: 0.14 M NaCl + 0.01 M Tris-HCl + 0.4 M sodium citrate</td>
<td>8.0</td>
<td>21.1, 13.1, 12.0</td>
</tr>
</tbody>
</table>

* The total protein concentration in each experiment was 5 mg per ml, the material was dialyzed against the solvent at 15° for 15 hours before ultracentrifuge analysis.

* Material was partially reduced and alkylated in sodium phosphate buffer, pH 7.5.

* Material was totally reduced and alkylated in 5 M guanidine-HCl, pH 7.6, and purified on a Sephadex G-200 column (Fig. 5).
of this range (Experiments C and D), there was dissociation of the 29 S component to components having lower sedimentation coefficients.

Reduction and alkylation of the purified hemagglutinin in 0.1 M sodium phosphate buffer, pH 7.5 (Experiment E), resulted in only minor dissociation of the major component and did not completely destroy the hemagglutination activity. As shown in Fig. 1D there was not a marked change in the sedimentation rate of components associated with hemagglutinin activity. Dialysis of the reduced and alkylated purified hemagglutinin against 5 M guanidine-HCl, pH 7.5, resulted in dissociation to a 4.8 S component (Experiment F). However, reduction and alkylation was not necessary to dissociate the major component to the lowest sedimenting subunit since dialysis against 5 M guanidine-HCl alone produced a 1.8 component (Experiment G). This value compares favorably with the 0.9 S value of the purified hemagglutinin subunit obtained by reduction and alkylation in 5 M guanidine-HCl at pH 7.6 (Experiment H). The reason complete dissociation was not obtained when material reduced and alkylated in phosphate buffer and then dialyzed against guanidine-HCl, pH 7.5 (Experiment F), may be caused by aggregation occurring during the reduction and alkylation process.

Dialysis against sodium citrate or EDTA resulted in loss of hemagglutinin activity. Since the major 29 S component can be partially dissociated by dialysis against 0.4 M sodium citrate (Experiment I), the role of divalent cations in stabilizing the structure of the molecule is strongly implicated.

Attempts were made to isolate the major hemagglutinin subunit so that further analyses could be made. To facilitate additional chemical studies the purified hemagglutinin was totally reduced and alkylated in 5 M guanidine-HCl prior to fractionation on upward-flowing Sephadex G-200 columns equilibrated with 5 M guanidine-HCl. A typical elution pattern is shown in Fig. 5 along with acrylamide gel electrophoresis patterns of the various pools of the peak. Pool 1 contained aggregates of material not entirely dissociated; Pools 2 and 3 contained a relatively homogeneous subunit with only traces of other material. Pool 2, which represents the major portion of the peak, was the material used for additional studies and will be referred to as purified hemagglutinin subunit.

Elution volume from a Sephadex G-200 column (Fig. 5) previously calibrated with molecules of known molecular weights, provided an estimate of the molecular weight of the purified hemagglutinin subunit. As suggested by Andrews (37) a plot of $\sqrt{K_o}$ versus $\sqrt{\text{mol wt}}$ was constructed from the values obtained and is shown in Fig. 6. Purified hemagglutinin subunit had a $K_o$ value of 0.481 which indicated a molecular weight of 20,000 ± 1,000.

### Table II

<table>
<thead>
<tr>
<th>Amino acid composition of purified hemagglutinin subunit</th>
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<tbody>
<tr>
<td><strong>Amino acid</strong></td>
</tr>
<tr>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td>Lysine</td>
</tr>
<tr>
<td>Histidine</td>
</tr>
<tr>
<td>Arginine</td>
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<tr>
<td>Aspartic acid</td>
</tr>
<tr>
<td>Threonine</td>
</tr>
<tr>
<td>Serine</td>
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<tr>
<td>Glutamic acid</td>
</tr>
<tr>
<td>Proline</td>
</tr>
<tr>
<td>Glycine</td>
</tr>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Valine</td>
</tr>
<tr>
<td>Methionine</td>
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<tr>
<td>Isoleucine</td>
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<tr>
<td>Leucine</td>
</tr>
<tr>
<td>Tyrosine</td>
</tr>
<tr>
<td>Phenylalanine</td>
</tr>
<tr>
<td>S-Carboxymethylcysteine</td>
</tr>
<tr>
<td>Tryptophan*</td>
</tr>
</tbody>
</table>

*Observed residues per molecule were calculated on the basis of an observed molecular weight of 20,000 less the weight due to carbohydrate (2,000).

* Determined spectrophotometrically.

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[Figures: CHYMOTRYPSIN and TRYPSIN]
The carbohydrate composition of purified hemagglutinin subunit was determined by descending paper chromatography of acid hydrolyzates of purified hemagglutinin subunit. This method revealed the presence of galactose, mannose, and glucosamine. Galactose and mannose made up 6.5% by weight of the molecule; glucosamine, the only hexosamine present, accounted for 6.6% of the weight.

The results of the amino acid analysis are given in Table II. It is noteworthy that lysine is absent from the molecule, but that rather large amounts of histidine and aspartic acid are present. Tracings of chymotryptic and tryptic peptide maps are shown in Fig. 7, A and B. The small number of peptides produced after trypsin digestion agrees with the small amount of arginine and the absence of lysine. As expected the 2 tryptophan residues determined spectrophotometrically were reflected by the two Ehrlich-positive peptides seen on the tracing of the chymotryptic peptide map. The NH$_2$-terminal amino acid analysis of oyster purified hemagglutinin subunit revealed threonine as the only NH$_2$-terminal group. Since these determinations were performed quantitatively, by the cyanate method of Stark (35), it is reasonable to assume that purified oyster hemagglutinin consists of a single polypeptide chain. This is further strengthened by the finding of a single residue of threonine at the NH$_2$ terminus and a single residue of alanine at the second position following Edman degradation.

**DISCUSSION**

A physical chemical analysis of oyster hemolymph has shown that there is one component, 33.4 S as determined by sedimentation velocity, which represents the majority of the total hemolymph components. The natural hemagglutinin appears to be associated with this molecule as determined by density gradient ultracentrifugal and gel filtration studies. Dissociation into slower sedimenting components can be brought about by dialysis against solvents at acid and alkaline pH values. Partial dissociation was also brought about by dialysis against sodium citrate. McDade and Tripp (36) have shown that calcium ions are necessary for the agglutination of human erythrocytes by oyster hemagglutinin as well as for the heat stability of the hemagglutinin molecule. These data suggest that calcium is important in stabilizing the structure of oyster hemagglutinin and probably explain the effect of sodium citrate on dissociation of the molecule. Complete dissociation to the subunit having a sedimentation coefficient of 1 S and a molecular weight of 20,000 could be brought about by dialysis against 5 M guanidine-HCl, pH 7.5. Thus, it is evident that the major macromolecular component of oyster hemolymph is a polymer of subunits noncovalently bonded. Furthermore, it would appear that only one class of subunit is involved since threonine was the only terminal amino acid residue detected. The close agreement between the amino acid composition and the number of peptides observed on peptide maps is additional evidence for a single type of subunit.

Recently a hemagglutinin from the horseshoe crab *Limulus polyphemus* has been isolated by Marchalonis and Edelman (42) so that a comparison between two invertebrate hemagglutinins is now possible. The horseshoe crab hemagglutinin is associated with a protein having a sedimentation coefficient of 13.5 S. This molecule also consists of a single class of subunit which has a molecular weight of 22,500. The subunit was not linked by covalent bonds since the 13.5 S molecule could be dissociated at high and low pH. The dissociation was enhanced by the presence of urea or guanidine HCl. Sodium citrate, as in the oyster hemagglutinin, was effective in partially dissociating the molecule. These and other data such as amino acid composition and peptide maps suggest a degree of structural similarity between oyster and horseshoe crab hemagglutinin. Whether these molecules represent primitive or precursor immunoglobulins as suggested by Burnet (17) cannot be determined from the limited sequence data available on the oyster hemagglutinin subunit. This possibility should not be definitely ruled out until more extensive sequence data have been collected for comparison with vertebrate immunoglobulins.

Acknowledgments—We acknowledge the many helpful discussions and criticisms of Drs. William Butler, John McKibbin, and Tetsuo Shiota. We express our gratitude to Karin Acton, Mary Hurst, and Freda Moore for their excellent technical assistance. Oysters were obtained through the courtesy of Lt. Gorton McCall of the Florida Board of Conservation, Panama City, Florida.

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J. Biol. Chem. 1969, 244:4128-4135.

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